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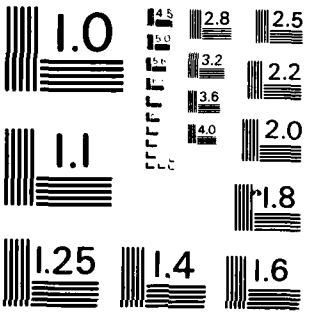

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The original problem was to characterize the effects of hallucinogenes, Ketamine and ditran, on endplate current (e.p.c.) parameters and to compare these with those of PCP. The model being used to establish uniform characteristics of these agents at the neuromuscular junction might be useful in understanding hallucinogenic drug action at other sites, particularly the brain. Ultimately, we planned to test methods of reversal of these effects at the neuromuscular junction which might be therapeutically useful in

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20. ABSTRACT CONTINUED:

reversing hallucinogenic manifestations clinically. In addition, we studied and characterized the interaction of several other classes of drugs (particularly irreversable acetylcholinesterase inhibitors) with the neuromuscular junction.

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1. (a) Statement of the Problem Studied

The original problem was to characterize the effects of hallucinogens, Ketamine and ditran on endplate current (e.p.c.) parameters and to compare these with those of PCP. The model being to establish uniform characteristics of these agents at the neuromuscular junction which might be useful in understanding hallucinogenic drug action at other sites particularly the brain. Ultimately, we planned to test methods of reversal of these effects at the neuromuscular junction which might be therapeutically useful in reversing hallucinogenic manifestations clinically. In addition, we studied and characterized the interaction of several other classes of drugs (particularly irreversable acetylcholinesterase inhibitors) with the neuromuscular junction.

(b) Summary of the Most Important Results

Ketamine (10 to 100 μM) and ditran (50 to 100 μM) reduced the peak current (Ip) and shortened the time course for endplate current (e.p.c.) decay (τ) in transected frog cutaneous pectoris muscles. Since ketamine has a pKa of 7.5, the block of e.p.c. parameters was more effective at pH 5.3 (more ionized) than at pH 9.1. Recovery from e.p.c. block by ketamine, ditran, and phencyclidine (PCP) was asymmetrical in that τ recovered more quickly than did Ip when the drugs were washed from the bath. Both ketamine and ditran disrupted the voltage dependence of τ, but unlike PCP, the relationship between Ip and membrane potential, although markedly depressed, was linear at all holding potentials. Ketamine and ditran blocked the binding of [^{3}H]-phencyclidine to microsacs from the electric organ of Torpedo treated with carbachol; IC_{50} = 6.6 x 10^{-6}M and 1.7 x 10^{-6}M, respectively. [^{3}H]-PCP binding reflects drug sites in the ion channel of the microsacs. The binding of [^{125}I]-alpha bungarotoxin to the microsacs was only slightly reduced by both compounds. Because of the asymmetry of e.p.c. parameters and the lack of interaction with α-bungarotoxin binding, it is suggested that ketamine and ditran block closed and open ion channels of the acetylcholine receptor and that recovery from block of closed channels (caused either by a direct action on closed channels or a very slow unblocking rate) proceeds more slowly than does the block of open channels.

Our initial studies of the effects of irreversible organophosphate acetylcholinesterase inhibitors on endplate currents (e.p.c.s.) have been completed and a preliminary report has been published. Three agents (ecothiophate, a tertiary methylamine analog and a tertiary ethylamine analog - tetram) were examined and found to be essentially equiactive in the range 1 to 100 μM. All three compunds (1-25 μM) decreased the rate of e.p.c. decay (α) and decreased the voltage dependence of α. The drug reduced α_0 (the decay rate at 0 mV) from 1.132 $msec^{-1}$ to 0.163 $msec^{1}$ and increased H (reflecting a decrease in the voltage dependence of α) from 111.6 mV to 756.4 mV. The effects on α and H were slowly reversible.

At higher concentrations (50-100 μM) the effects of ecothiophate on the absolute magnitude of α were reduced while the decrease in voltage dependence was the same as for lower concentrations. In contrast, the tertiary methylamine analog caused biphasic e.p.c. decay (V_m = -70 to -130 mV). Similar effects of both ecothiophate and the tertiary methylamine analog were observed on miniature e.p.c.s in intact muscle.

The effects of higher concentrations (50-100 μM) of these drugs reflect actions on the acetylcholine receptor ionic channel in addition to the block of acetylcholinesterase. The effects of all concentrations on H are less readily associated with a direct effect on the ionic channel and may be mediated through other effects of these agents. In order to test the possibility that the effects on H were due to the increased bombardment of the receptor with acetylcholine, we decreased transmitter release with Mg^{++} or decreased the number of functional receptors with α-Bungarotoxin, both in the presence of the inhibitor. In both situations the ability of the agent to affect H was unmodified.

The effects of hemicholinium-3 (HC-3), hemicholinium-15 (HC-15, the monoquaternary half molecule of HC-3) and p-terphenyl-hemicholinium-3 (TPHC-3, an analogue with a terphenyl rather than biphenyl nucleus) on endplate currents (e.p.c.s) in transected frog cutaneous pectoris muscles were examined. At holding potentials (V_m) more negative than -90 mV, HC-3 caused a monotonic increase in the rate of e.p.c. decay (α; V_m = -90 mV, control α = .61 ± .04, S.E. $msec^{-1}$ and with HC-3, 50 μM, α = 1.75 ± .31 $msec^{-1}$). At less negative V_m's in the presence of HC-3, e.p.c. decay was biphasic, the initial phase being faster and the final phase slower than control. At V_m = -30 to -10 mV, the relative amplitude of the terminal phase equaled that of the initial fast phase. At V_m = +30 mV, a single slow exponential decay was observed (control α = 1.77 ± .10 $msec^{-1}$ and with 50 μM HC-3 α = 0.60 ± .11 $msec^{-1}$). Over the range of V_m = -10 to -20 mV $\alpha_{slow} \cong 0.3$ $msec^{-1}$ and $\alpha_{fast} \cong 1.45$ $msec^{-1}$ were independent of V_m. Peak e.p.c. amplitude (Ip) was depressed at all V_m's but proportionally more depressed at V_m's more negative than -30 mV. Similar results were obtained with miniature e.p.c.s in intact muscles and Mg^{++}-depressed intact muscles. HC-15 (50-200 μM) caused a monotonic slowing of e.p.c. decay at all V_m's with accentuation of the normal voltage dependence of α. HC-15 (100 μM) increased the voltage dependence (H) of α from control values of -149 ± 13 mV to -107 ± 17 mV. Ip was slightly depressed by HC-15 at all V_m's. All effects of both HC-3 and HC-15 were rapdily reversible. TPHC (5-100 μM) caused a time and concentration dependent increase of α and decrease in Ip. With 25 μM TPHC-3, not at a steady state, α was increased and Ip was depressed at all V_m's. The effects of TPHC-3 on e.p.c. parameters were slowly reversible with the recovery of α faster than Ip. The effect of these drugs on e.p.c. kinetic parameters suggest that they interact in diverse fashions with the acetylcholine activated ionic channel.

The effects of hemicholinium-3 (HC-3) and HC-15 (the monoquaternary half-molecule of HC-3) on single AcCho-activated ionic channels in embryonic chick myotubes were examined. At the resting potential (V_m = -40 to -60 mV) single channel conductance was unaltered by HC-3 (25-80 μM) but beam channel open time (t_o) was decreased in a concentration dependent manner [(t_o(control) = 6.6 ± 0.4 msec; t_o (HC-3, 25 μM) = 3.0 ± 0.2 msec; t_o (HC-3, 50 μM) = 1.7 ± 0.1 msec; t_o (HC-3, 80 μM) = 1.16 ± 0.08 msec)]. An estimated equilibrium dissociation constant (K_D) of the block of open channels of 2.2 x 10^{-6} M was obtained. When V_m was set at +20 mV, HC-3 (50 μM) prolonged t_o from 0.7 msec to 3.0 msec. In contrast to HC-3 at V_m = -40 to -60 mV, HC-15 (10 μM) prolonged t_o from 6.2 ± 0.3 msec to 12.4 ± 0.9 msec. Higher concentrations of HC-15 (50-100 μM) initially prolonged single channel currents and, within a few seconds, caused prolonged bursts of brief openings and closings, suggesting open channel blockade. The K_D's of open channel block by HC-15 were 37 μM

(V_m = -100 mV) and 17 μM (V_m = -50 mV) i.e. opposite voltage dependence than typical channel blocking agents. These data, in conjunction with those obtained in a companion study, suggest at least two sites of action of these compounds on the AcCho receptor-channel complex, one to block open channels and one to prolong channel open time.

Our initial studies involving the examination of hallucinogenic agents on esterase poisoned endplates have been completed and a preliminary report has been submitted for published. In endplates treated and washed for several hours with the aforementioned irreversible esterase inhibitors, there was only a slight reversal of the slowing of e.p.c. decay and the reduction of the voltage dependence of α (decay rate $msec^{-1}$). The addition of atropine (10 μM, a concentration having no effect on α in untreated preparations) after esterase inhibition greatly accelerated e.p.c. decay (α_0 = α at V_m = 0 = 0.266 $msec^{-1}$, before and 0.652 $msec^{-1}$ after the addition of atropine. The interruption of the voltage dependence of α by the esterase inhibition was further accentuated by atropine. Likewise, the effects of ketamine (30 μM) and phencyclidine (PCP) to increase α and interrupt voltage dependence were greatly accentuated after irreversible esterase inhibition. Other agents which prolong e.p.c. decay (e.g. ethanol) by a mechanism not involving esterase inhibition, do not potentiate the channel blocking actions of atropine or hallucinogens.

[^{3}H]PCP binding to the acetylcholine-receptor channel is a reliable measure of interaction at a site or sites different than the receptor recognition site. In order to test whether irreversible esterase inhibition altered the ability of atropine and ketamine to interfere with this binding, we tested [^{3}H]PCP binding to torpedo microsacs. The IC_{50}'s for ketamine and atropine for block of [^{3}H]PCP binding were not different whether the esterase was active or inactive. It is suggested from these studies that the enhanced effectiveness of these drugs to block open ACh receptor channels is due to the increased frequency of channel opening. To test this hypothesis, studies are currently underway with purified ACh-receptor channels incorporated into planar lipid bilayers. In this system, single ACh-activated channel currents can be measured without acetylcholinesterase present and with controlled levels of agonist.

We have also characterized the effects of agents which are thought to antagonize the central actions of hallucinogenic agents. Tacrine has been reported to antagonize some of the behavioral effects of these agents. At low concentrations (< 10 μM) tacrine prolongs e.p.c. decay and eliminates the voltage dependence of α similar to the ecothiophate analogs and consistent with its ability to inhibit acetylcholinesterase reversibly. At higher concentrations (≥ 25 μM), tacrine causes biphasic e.p.c. decay with holding potentials (V_m) between -30 and -130 mV, with the fast phase of decay faster than control and the slow phase slower. For positive V_m's only slowing of e.p.c. decay occurred. The speeding of e.p.c. decay at these higher concentrations is indicative of ACh-activated ionic channel blockade and like that caused by 217A0. An analogue of tacrine, 9-aminoacridine, which differs in the number of the double bonds of one of the three rings, reverses the normal voltage dependence of α. That is, e.p.c.s are much faster than control at negative V_m's and greatly slowed at positive V_m's. The reasons for these differences are still to be determined

c. List of All Publications and Technical Reports Published

1. Ketamine and ditran block endplate ion conductance and [^{3}]-phencyclidine binding to electric organ membrane. R.L. Volle, K.A. Alkadhi, D.D. Branisteanu, L.S. Reynolds, P.M. Epstein, H. Smilowitz, J.J. Lambert, and E.G. Henderson. J. Pharmacol. Exp. Ther. 221:570-576, 1982.

2. Comparative aspects of nicotinic receptor blockade by ketamine, ditran and lobeline. C.J. Smith, P.M. Epstein, J.J. Lambert, R.L. Volle, and E.G. Henderson. Fed. Proc. 41:4924a, 1982.

3. Drug-induced modification of ionic conductance at the neuromuscular junction. J.J. Lambert, N.N. Durant, E.G. Henderson. Ann. Rev. Pharmacol. and Toxicol. 23:505-539, 1983.

4. Ecothiophate (217MI) and its tertiary amine analogue (217AO) decrease voltage dependence of endplate current (e.p.c.) decay in frog skeletal muscle. E.G. Henderson, R.L. Volle, L.S. Reynolds, J.J. Lambert, and P.M. Epstein. Fed. Proc. 42:991, 1983).

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6. The hemicholiniums. I. Postsynaptic nicotinic receptor interactions revealed by alteration of endplate current kinetics. E.G. Henderson, R.L. Volle, H. Smilowitz, L.S. Reynolds, K.A. Alkadhi, J.J. Lambert and M.L.J. Ashford. (In revision.)

7. The hemicholiniums. II. Direct interactions with nicotinic acetylcholine receptor channel of the chick myotube revealed by single channel measurements. M.L.J. Ashford, J.J. Lambert, R.L. Volle and E.G. Henderson. (In revision.)

8. Potentiation of acetylcholine receptor-channel blockade by atropine and hallucinogens after irreversible acetylcholinesterase inhibition with organophosphate agents. E.G. Henderson, L.S. Reynolds, R.L. Volle, S. Moraski Jr. and P.M. Epstein. Soc. for Neurosci. Abstr. (in press, Vol. 9:456, 1983).

9. Calcium channel antagonists displace [^{3}H]phencylidine binding to torpedo electric organ membrane. P.M. Epstein, J.J. Lambert, S. Moraski Jr. and E.G. Henderson. Soc. for Neurosci. Abstr. (in press, Vol. 9:735, 1983).

10. Direct interactions of the hemicholiniums with the nicotinic acetylcholine receptor channel revealed by single channel current measurements. E.G. Henderson, R.L. Volle, J.J. Lambert, and M.L.J. Ashford. Proceedings of 1982 Chemical Defense Research Conference, J. of Res. Comm., 1983, spec. public. ARCSL-5P-83030.

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